We claim:

1. A method for the amplification of RNA, in a sample, comprising:

a) obtaining a starting solution by adding to a container comprising the sample, a buffer, a first primer, a second primer, a plurality of nucleotide triphosphates, a sufficient amount of an enzyme system having reverse transcriptase activity and a heat stable enzyme system having DNA polymerase activity, and

closing the container, wherein said sufficient amount is an amount which, after the heat treatment of step b) below, will retain sufficient reverse transcriptase activity to permit performance of step c) hereafter;

b) heating the solution obtained in a) to a temperature sufficient to permit denaturation, said temperature not to exceed 75° C., and maintaining said temperature for a sufficient time to provide denaturation of said RNA without inactivating the enzyme system having reverse transcriptase activity;

c) bringing the solution obtained in b) to a predetermined temperature and maintaining said temperature for sufficient time whereby a first cDNA strand is synthesized and a RNA-cDNA heteroduplex is formed;

d) heating the solution obtained in c) to a predetermined temperature whereby said RNA-cDNA heteroduplex is denatured to form an RNA single strand and a first cDNA single strand;

e) bringing the solution obtained in d) to a predetermined temperature and maintaining said temperature for a sufficient time whereby the second primer hybridizes with the first cDNA strand;

f) bringing the solution obtained in e) to a predetermined temperature and maintaining said temperature for a sufficient time whereby a second cDNA strand is synthesized to form a double-stranded cDNA; and

g) denaturing the double-stranded cDNA and subjecting the cDNA strands to a sufficient number of amplification cycles to obtain a desired amount of amplified product.

2. The method as claimed in claim 1, wherein in step b) said solution is heated to at least 60° C.

3. The method as claimed in claim 1, wherein in step c) said predetermined temperature is a temperature which permits the hybridization of the first primer to said RNA without permitting hybridization of the primer to an RNA sequence that is not absolutely complementary.

4. The method as claimed in claim 1, wherein prior to step c) said solution obtained in b) is brought to a temperature which permits the hybridization of the first primer to an RNA sequence which is not absolutely complementary, said temperature being at least 40° C. and lower than 50° C.

5. The method as claimed in claim 1, wherein in step c) said predetermined temperature is between 50° and 65° C.

6. The method as claimed in claim 5, wherein in step c) said predetermined temperature is between 50° C. and 60° C.

7. The method as claimed in claim 1, wherein in step c) said sufficient time is less than about 15 minutes.

8. The method as claimed in claim 1, wherein said enzyme system having reverse transcriptase activity is selected from the group consisting of avian myoblastosis virus and Moloney murine leukemia virus.

9. The method as claimed in claim 1, wherein the amounts of reverse transcriptase activity and of DNA polymerase activity are such that the ratio of units of reverse transcriptase to units of DNA polymerase is from 2 to 8.

10. The method as claimed in claim 9, wherein said ratio is from 2 to 6.

11. The method as claimed in claim 1, wherein in step d) said predetermined temperature is above 90° C.

12. The method as claimed in claim 1, wherein in step e) said predetermined temperature permits the hybridization of the second primer without permitting hybridization of the second primer to a DNA sequence that is not absolutely complementary.

13. The method as claimed in claim 1, wherein in step e) said predetermined temperature permits the hybridization of the second primer to a DNA sequence which is not absolutely complementary, said temperature being at least 40° C. and lower than 50° C.

14. The method as claimed in claim 1, wherein in step f) said predetermined temperature maintains the hybridization of the second primer.

15. The method as claimed in claim 1, wherein said sample is deposited on or attached to a support.

16. The method as claimed in claim 1, for the amplification of human immunodeficiency virus RNA, wherein the first primer comprises the following sequence:

CCTATCTGTCCCCTCAGCTAC (SEQ ID NO: 1).

17. The method as claimed in claim 1, for the amplification of human immunodeficiency virus RNA, wherein the second primer comprises the following sequence:

TCTATCAAAGCAACCCAC (SEQ ID NO: 2).

18. The method as claimed in claim 14, wherein in step f), said predetermined temperature is at least 50° C.

19. The method according to claim 1, wherein said RNA to be amplified contains or is capable of containing secondary structure.

20. The method according to claim 1, wherein a sufficient time to provide denaturation of said RNA ranges from 1 minute to 15 minutes.

21. The method as claimed in claim 1, wherein in step c) said predetermined temperature is at least 45° C.

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22. A method for the amplification of RNA in a sample in a closed container, comprising:

a) obtaining a starting solution by placing, in a container, the sample, a buffer, a first primer, a second primer, a plurality of nucleoside triphosphates, and an enzyme system having reverse transcriptase activity and DNA polymerase activity, and closing the container;

b) heat treating said solution at a temperature sufficient to permit denaturation of secondary structures that may be present in said RNA but not above 75° C., for a time sufficient to permit denaturation of secondary structures without completely inactivating the reverse transcriptase and DNA polymerase activities of said enzyme system;

c) permitting a first cDNA strand to be synthesized and a RNA-cDNA heteroduplex to be formed;

d) heat treating the solution containing said RNA-cDNA heteroduplex at a temperature at which said heteroduplex is denatured to form an RNA single strand and a first cDNA single strand without completely inactivating the DNA polymerase activity of said enzyme system;

e) permitting the second primer to hybridize with the first cDNA strand, followed by synthesis of a second cDNA strand to form a double-stranded cDNA; and

f) denaturing the double-stranded cDNA and subjecting the cDNA strands to a sufficient number of amplification cycles to obtain a desired amount of amplified product.

23. The method of claim 22, wherein said enzyme system comprises at least one first enzyme having reverse transcriptase activity and at least one second enzyme having DNA polymerase activity.

24. The method of claim 23, wherein said at least one first enzyme comprises at least one enzyme selected from the group consisting of RT-AMV and RT-MMuLV.

25. The method of claim 23, wherein said at least one first enzyme comprises RT-AMV and said at least one second enzyme comprises Taq polymerase.

26. The method of claim 22, wherein said temperature sufficient to permit denaturation of secondary structures that may be present in said RNA is from about 60° C to about 75° C.

27. The method of claim 26, wherein said first cDNA strand is synthesized and said RNA-cDNA heteroduplex is formed at a temperature from about 50° C to about 75° C.

28. The method of claim 27, wherein said temperature at which said heteroduplex is denatured is above 90° C.

29. The method of claim 28, wherein the second primer is permitted to hybridize with the first cDNA strand at a temperature from about 50° C to about 80° C.

30. The method of claim 29, wherein said synthesis of said second cDNA occurs at a temperature from about 50° C to about 80° C.

31. The method of claim 22, wherein said first cDNA strand is synthesized and said RNA-cDNA heteroduplex is formed at a temperature from about 45° C to about 75° C.

32. The method of claim 31, wherein said first cDNA strand is synthesized and said RNA-cDNA heteroduplex is formed at a temperature from about 50° C to about 65° C.

33. The method of claim 22, wherein the second primer is permitted to hybridize with the first cDNA strand at a temperature from about 50° C to about 80° C.

34. The method of claim 22, wherein the said synthesis of said second cDNA strand occurs at a temperature from about 50° C to about 80° C.